PAMPering immune responses : spotlight on helper cells for dendritic cell vaccination

8 Valorisation

Cancer is one of the leading causes of death worldwide. In 2012, 14.1 million new cancer cases (101.210 cases in NL) and 8.2 million deaths (43.666 deaths in NL) have been registered with the majority of both incidence and mortality reported in less developed countries [1-3]. The number of new cases is expected to rise by 70% in the upcoming 20 years to 24 million [4]. In 2008, an estimated 83 million years of 'healthy life' were lost due to early death and disability caused by cancer. This implies an immense economic and social burden. Besides the direct costs for cancer treatment, also these indirect costs due to morbidity are included in the economic burden. As such, cancer accounts for the biggest economic impact worldwide among the leading causes of death [5]. Moreover, cancer diagnosis does not only affect the patients themselves but also their families and caregivers, and thus has an even broader physical, psychosocial, and economic impact [6].

Early detection and current treatment strategies, such as surgery, radiotherapy and chemotherapy have been improved substantially during the last decades, leading to a decline of mortality. Nonetheless, a considerable high number of cancer cases remain incurable. Besides further improving screening methods as well as current anti-cancer treatments, there is need for developing innovative less invasive and non-toxic treatment options to ensure a better quality of life for patients with cancer.

The field of immunotherapy has gained particular interest among researchers for the development of novel anti-cancer treatment strategies. The general approach is to use or stimulate the patient's immune system in order to fight the cancer. Several approaches have been approved by the American Food and Drug Administration (FDA) and are currently implemented as part of standard anti-cancer treatment regimens for specific types of cancer. Different types of cancer immunotherapy exist, which can be divided into passive and active approaches as well as specific and non-specific mechanisms of action. One approach of cellular immunotherapy are dendritic cell (DC)-based vaccines. The use of DC as therapeutic vaccine is based on their primary role in detecting invading pathogens in our body. The principle of this approach is to isolate white blood cells of the patient and isolate a precursor of these cells (monocytes) and manipulate them *ex vivo*. This includes instructing them to recognize specific pattern expressed only by the cancer cells and boosting their capacity by adding specific triggers to maximize their potency, the so-called maturation stimuli. DC-based vaccines are already tested in clinical trials and have been proven to be safe and non-toxic, however overall clinical outcome is still limited. The first vaccine against advanced prostate cancer, Provenge®, has been approved by the FDA in 2010 [7, 8]. This vaccine is prolonging the patient's life-time for several months without eradicating the tumour. Moreover, several clinical trials are currently ongoing testing various DC-based vaccination strategies illustrating the potential role of DC-based vaccines in future standard anti-cancer treatments. Nonetheless, the limited overall positive clinical outcome monitored

thus far is indicative for the need of further optimizations.

This thesis aimed to improve these therapeutic anti-cancer DC-based vaccines. We focused on analysing the potency of different maturation stimuli on the capacity of DC to interact with helper cells of the immune system (T helper cells and natural killer cells) which is important to eliminate cancer cells.

We revealed that, at least *in vitro*, the potency of a dendritic cell to interact with natural killer cells and T cells is dependent on their capacity to produce one soluble factor: the cytokine IL-12. In the human system, this factor dose-dependently determines the outcome of the immune response and thus the potential anti-cancer response. These findings are strengthened by recent clinical trials indicating a positive correlation between high IL-12-producing DC and time to disease progression [9, 10]. Moreover, older studies tested the systemic application of IL-12 and revealed a positive anti-cancer effect. However, this implementation of systemic IL-12 administration in cancer treatment approaches was hindered by its dose-limiting toxicities [11-17]. Altogether, these findings emphasize on the use of IL-12-producing DC to ensure local production and delivery of this cytokine in order to come one step closer to successful vaccination strategies. This knowledge is very important to set new release criteria for DC maturation stimuli.

We showed that stimuli which have been used thus far by other groups did not lead to high IL-12-producing DC. However, we also revealed a huge donor-to-donor variation in the capacity of DC to produce IL-12 upon the same stimulation. Further research should investigate whether e.g. polymorphisms in the IL-12 gene are responsible for this in order to select only responding patients for clinical trials. Alternatively, DC-derived from low-responders could be engineered to constitutively produce high levels of IL-12. With introducing this new release criterion there is a possibility to increase the currently moderate clinical responses of anti-cancer DC-based vaccination.

In this line, we identified a maturation cocktail which is able to generate high IL-12-producing DC. This combination of maturation stimuli has been filed as patent in May 2012 [18]. Consequently, in March 2015, a spin-off company, CiMaas, has been founded by Prof. dr. GMJ Bos (CEO) and Dr. WTV Germeraad (CSO) being two of the inventors of the intellectual property. This biotech company focuses on the development of cellular immunotherapy against cancer. The target group for DC-based vaccines in initial clinical trials will be patients with lung cancer (5-year survival in NL: 17%) and patients with multiple myeloma (5-year survival in NL: 40%) [19]. During the upcoming two years the knowledge acquired in this project as well as preceding research by our group will be translated into the start of a clinical trial. Currently, the findings are validated and tested,

which also includes regulatory affairs. All products need be available in good manufacturing practice (GMP) and standard operating procedures need to be set up. After the writing of the investigational medicinal product dossier and the permission of central committee on research involving human subjects, two phase I/II clinical trials are scheduled to start in the first quarter of 2017. DC made according to the procedure described in *chapter 4* will be electroporated with mRNA coding for the Wilms-tumour I antigen, an important tumour antigen expressed in cancer cells and being essential for their survival. A collaboration with Prof. dr. K. Thielemans (University of Brussels, Belgium) has been set up from whom the mRNA will be supplied. The design of the trial is similar to the design of trials by Dr. V.F.I. van Tendeloo (University of Antwerp, Belgium) to allow some comparison of the different DC in a clinical context.

Even though the introduction of Provenge® to the market shows the potential of personalized cell therapy as new anti-cancer treatment, several aspects and patient inclusion criteria may be considered before starting the clinical trials with the optimized method to generate DC as described above. To prove the potential superiority of our new DC generation method, in initial trials only patients of which the DC have high IL-12-producing capacities determined on beforehand *in vitro* should be included. Once the success of this treatment has been proven, alternatives to also treat low-responders should be developed.

Another crucial hurdle for the effectiveness of cellular immunotherapy in general is the suppressive tumour environment. The tumour is embedded in a complex microenvironment formed by lymphoid myeloid cells, stromal cells, vasculature, lymphatic vessel, cytokines and chemokines. The tumour itself can interfere by the expression of inhibitory ligands, creation of a tolerogenic environment, and recruitment of regulatory cells [20,21]. Tumours are able to co-opt immune inhibitory pathways which are under physiological circumstances responsible to regulate immune responses and control self-tolerance, duration, and magnitude of the induced responses. As such tumours increase their immune resistance. To increase the efficacy of DC-based vaccination, combination therapies should be applied, targeting the tumours on multiple levels by using e.g. checkpoint inhibitors.

Besides the implementation of improved DC-based vaccines, CiMaas is also focussing on NK cell therapy. NK cells are important in killing virally infected cells of the human body and also play a crucial role in killing tumour cells. This adoptive cell therapeutic approach is based on the use of donor NK cells, selected according to specific criteria, to attack the cancer cells. In humanized mouse models for breast cancer and multiple myeloma, NK cells eliminated the tumours. CiMaas is currently focusing on methods to generate high numbers of NK cells in GMP complying conditions which are needed for translation of this therapy to cancer patients. Optimizations aim to enhance the cytotoxic capacity of NK cells by

addition of various cytokines during expansion and the selection of appropriate allogeneic donors having KIR-ligand mismatched NK cells favouring their cytotoxic capacities. Even though studies described in this thesis where not primarily focusing on improving NK cell therapy, we did show that direct incubation of NK cells with pathogen-derived products (PAMPs) positively influenced the activation of NK cells and subsequent interaction with DC. Moreover, increasing evidence in literature describes enhanced cytolytic capacities of NK cells after PAMP-activation [22, 23]. Incorporation of PAMPs to activate NK cells before injection could enhance the NK cell-mediated effects *in vivo* and may reduce the number of NK cells needed to treat a patient. Arguably, NK cells could also be injected with adjuvants in order to boost their activity *in vivo*. Further research is needed to evaluate the effect of these pathogen-derived products on NK cell function.

To sum up, the findings described in this dissertation mainly contribute to the progress of therapeutic DC-based anti-cancer vaccines. The obtained data of this thesis and previous research efforts of our laboratory are currently translated into clinical products and will be soon applied in clinical trials. Possibly, this form of personalized treatments will in the near future not only achieve prolongation of the patient's life-time but also attack the tumour itself more efficiently in non-toxic manner and be incorporated more and more in future standard anti-cancer treatment regimens.

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